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to H5 could explain in part its wider distribution. All the haplotypes found belong to the haplogroup A, indicating that the analysed populations derive from the same maternal lineage emerged after expansion from isolated glacial refugia, according to phylogeographic structure common to many freshwater fish. As for the genetic variability, estimated by the haplotype (H) and nucleotide (π) diversity, NIN and SEL were monomorphic, while IRM, PRA and ALC showed a considerable level of variability (H: $0.60 \div 0.67$; π: $0.029 \div 0.037$). In general, the Sicilian populations exhibit a higher haplotype diversity compared to the other Italian populations studied until now (mean H value: 0.36 *vs* 0.26), while show similar values for the mean π value (0.018 *vs* 0.020). Stocking from outside Sicily could have contributed to the high variability of some Sicilian populations.

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P005

Lipofection conditions of CHO-K1 cells with the use of two transposon systems

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This study aimed to elaborate CHO-K1 cells transfection conditions with the use of two transposon systems: piggyBac and Tol2 by lipofection method. Firstly, we optimized transfection conditions for Tol2 transposons. We compared effect of cells density (1×10^5 cells/ml and 5×10^5 cells/ml), amount of lipofectant Xtreme HP DNA (1-8µl) and plasmid constructions: pCMV-Tol2 with pL-miniTol2-OVA5IFN, pCMV-Tol2 with pL-miniTol2-OVA5Egfp and pL-OG-OVAIFNEnh-Egfp (control vector) on viability of cells. To select transfected cells we used G418 at concentration 400µg/ml, which was determined based on antibiotic kill curve. The viability of cells was the marker of transfection efficiency and was examined with the use of flow cytometry FACS Aria after iodide propidium (PI) staining. Secondly, using the most effective conditions of lipofection, we transfected cells with plasmids piggyBac transposons: pCMV-sPBo (Transposagen) with pLPB-NeoOVA5fEIFN and pCMV-sPBo with pLPB-NeoOVA5fEegfp. Significant differences in the viability of transfected CHO-K1 cells were calculated with a three-factor analysis of variance followed by Bonferroni and Games-Howell tests.

The highest transfection efficiency was obtained for concentration 1×10^5 cells/ml. In comparison to control (32%), the highest lipofection efficiency for Tol2 transposon vectors was achieved for cells transfected with 0.5µg pCMV-Tol2 with 1µg pL-miniTol2-OVA5IFN and 0.5µg pCMV-Tol2 with pL-miniTol2-OVA5Egfp and 1µl Xtreme HP DNA (62.0 and 60.1%, respectively). The same conditions of lipofection used to transfect the cells for pCMV-sPBo with pLPB-NeoOVA5fEIFN and pCMV-sPBo with pLPB-NeoOVA5fEegfp resulted in cells viability 76.8 and 77.9%, respectively. The percentage of live cells after transfection with the use of control vector at density 1×10^5 cells/ml and 5×10^5 cells/ml was 67.3% and 36.7%, respectively. The EGFP expression after transfection for 1×10^5 cells/ml and 5×10^5 cells/ml was 32.3 and 11.2%, respectively. These elaborated transfection conditions will be used to transfect chicken primordial germ cells (PGCs) in the next step of our project.

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P006

Genetic variability detected at the (c-type) milk lysozyme encoding gene in donkey

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Lysozyme is known to be a natural antimicrobial agent since it catalyses the hydrolysis of glycosidic bonds of mucopolysaccharides in bacterial cell walls. It inhibites the development of many pathogens bacteria, thus making the milk somewhat selective in regards to the milk bacteria content. Three major distinct types of lysozymes have been identified: chicken-type (c-type), invertebrate-type (i-type), and goose-type (g-type). In particular, there are at least 4 non-stomach lysozyme genes in ruminants (i.e., mammary gland, kidney, trachea, intestinal). Lysozymes in ruminants and equine milk are

considered to be the c-type because of their similarity to chicken egg white lysozyme. The c-type lysozyme content in donkey's milk varies during the different stages of lactation with a mean value of 1.0 mg/mL and proved to be higher than that in bovine, ovine, caprine (traces), whereas it was very close to mare's milk. In the equine species, the c-type lysozyme encoding gene (4 exons) maps on chromosome 6 and transcribes a mRNA of 1329bp, coding for a protein of 148aa. To our knowledge, no information on genetic variability has been reported so far at this *locus* in donkey. Consequently, in order to detected variability, total RNA was extracted from milk somatic cells of 6 unrelated Ragusana donkeys reared in Central Italy. The mRNA fragment comprised between the last 84nt of exon 1 and the first 285nt of exon 4 was amplified by RT-PCR and sequenced. Primers (For GCAAGGTCTTTG-AAAGATGT and Rev ACCAGCATTAGTTCTATTCG) were designed using as template the genomic donkey sequence (EMBL ID: NW_014638180). The obtained sequence (465bp) is relative to the cDNA tract spanning the last 64nt of exon 1 to the 236thnt of exon 4. Stop codon is located at the 65th-67thnt of exon 4. Sequences comparison showed a transition G→A at the 160thnt of exon 2 (NW_014638180:g-1784688C > T) responsible for the aa change Arg^{90}→Gln. The presence of the codon CGA at exon 2 of the donkey milk lysozyme encoding gene might represent the ancestral condition of the gene in *equidae*, as it has also been found in other donkey and male sequences. The identification of this SNP could represent the first report of polymorphism at this *locus* in donkey. Next step of the research will be the analysis of a large number of samples in order to establish the frequency of this mutation in donkey species and to evaluate if and how the new genetic variant may influence functional and biological properties of donkey's milk.

P007

First SNP discovery in ACACA gene and association study with milk yield in Mediterranean river buffalo

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Table 1 Genotyping data and effects of *ACACA* SNP genotypes on milk yield.

Locus	Trait	Genotype		
ACACA	Genotype distribution	CC (430)	CT (112)	TT (9)
	N. lactations	854	223	19
	N. records	5603	1498	135
	Average milk quantity	8.36	8.18	7.32

The *ACACA* enzyme catalyses the first committed step of fatty acid synthesis in mammalian cytosol, the carboxylation of acetyl-CoA to malonyl-CoA, leading to the biosynthesis of long-chain fatty acids. To our knowledge no information on DNA genetic variability at *ACACA locus* has been reported so far in buffalo species. Consequently, in order to detected polymorphisms at Italian Mediterranean river buffalo *ACACA locus* and test possible associations with milk yield, we analyzed 551 subjects belonging to 14 farms, located in Salerno and Caserta province. A total of 7096 records for milk yield measured monthly with an automatized milk recording system on 1096 lactations were used. The DNA regions of the *ACACA* gene spanning partial exons 1 of 10 individual samples, randomly chosen, were amplified and sequenced using primers (GACAGTTTCTGACCTTTTGGTG and AGACCTCTCTG-CTTCCAA) designed on the genomic buffalo sequence (EMBL acc. no. NW_005785166). Sequence comparison showed a transvertion C→T at position 34 of the exon 1 (5' UTR) (NW_005785166:g4381303G > A). The genotyping of DNA samples was performed at the KBiosciences (http://www.kbioscience.co.uk) laboratory. The major allele had a relative frequency of 0.88 and the *locus* was in Hardy–Weinberg equilibrium. A mixed linear model procedure of SAS 9.1 (SAS Institute) was used for the association analysis between genotypes and milk yield. The model included fixed effects of the genotype, farm, calving season, days in milk, parity and the random effect of the animal. None of the three genotypes had significant associations with milk yield (Tab. 1). In conclusion, in this study, we report the first SNP identification at the *ACACA locus* in Mediterranean river buffalo. Although we found no association between the detected polymorphism and milk yield, our work provides a starting point for studies of the future possible association between *ACACA* variation and other milk phenotypic traits in buffalo.

P008

The interleukin-10 polymorphism g.3936 G > A is uncoupled with bovine tuberculosis susceptibility in water buffalo (*Bubalus bubalis*)

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